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In vitro evaluation of antifungal activity of Medicinal Plant extracts against Foliar Pathogens of Japanese Mint (*Mentha arvensis*)

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ABSTRACT

Extracts of eight plants namely neem, tulsi, kalmegh, vasaka, papaya, sanjeevani, giloy and moringa were evaluated *in vitro* by poisoned food technique at 5%, 10% and 15% concentration against *Alternaria alternata* and *Curvularia lunata* causing leaf blight and leaf spot disease in mentha respectively. All the three concentrations of eight plants extracts inhibited radial growth of pathogen significantly compared to control. Among these plant extracts, papaya leaf extract performed best by showing 62.80%, 74.38% and 81.26% mycelial growth inhibition of *Alternaria alternata* at 5%, 10% and 15% concentration respectively. Mean percent inhibition of mycelial growth of *Alternaria alternata* with papaya extract was recorded 72.82% which was recorded highest among all other plant extracts. The lowest mean percent inhibition of mycelial growth of *Alternaria alternata* over control was observed in case of vasaka extract (33.99%). All the three concentrations (5%, 10% and 15%) of papaya extract also found most effective in mycelial growth inhibition of *Curvularia lunata* i.e., 59.81%, 68.9% and 90.43% respectively. Similarly, highest mean percent inhibition of mycelial growth of *Curvularia lunata* was recorded in papaya extract (73.05%) followed by kalmegh extract (58.85%). Least mean inhibition percentage of the mycelial growth of *Curvularia lunata* was found with giloy extract (22.49%).

Key words: *Plant extracts, Alternaria, Curvularia, Mentha, Papaya, Kalmegh*

Introduction

Mentha arvensis commonly called Japanese mint is an aromatic perennial herb belongs to family Lamiaceae and is commercially cultivated in Tropical and Subtropical climates. Mints are widely cultivated on almost all types of soils and climates. China, India, USA, Japan, France, Italy, Russia and Bulgaria are some of the major producers of mint oils (Kalra *et al.*, 2004). There have been 30 or more pathogens recorded on mints. The intensity of damage caused by various organisms varies according to topography and climatic condition. Foliar diseases such as Alternaria leaf blight causes heavy defoliation of mentha leaves simultaneously reduces essential oil yield (Shukla *et al.*, 2000). Leaf spot and blight cause significant yield losses primarily in Bihar and Bengal region of India. Thakur *et al.* (1974) first time reported *Curvularia lunata* causes leaf spot of mentha was from India. Mentha contains essential oil which is used in pharmaceutical industries, hence for management of mentha diseases, there is a need to have inexpensive and environmentally safe practices. In the present era, phytoextracts could be one of the

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alternativeeco-friendly disease management approaches. Sharma *et al.,* (2021) screened seven plant extracts against *Alternaria alternata* causing Alternaria blight of tomato and found that both 10% and 15% concentration of garlic (*Allium sativum*) extract performed best in mycelial growth inhibition (100%) followed by neem extract (*Azadiracta indica*). Akinbode (2010) evaluated four medicinal extracts against *Curvularia lunata* causing leaf spot of maize and it was observed that at all concentrations, *Phyllanthus amarus* was most effective followed by extract of *Tithonia diversifolia* and *Morinfa lucida*. Tremendously depending on chemical fungicides for controlling plant diseases leads to cause hazardous effects on environment and simultaneously develops resistance in pathogen to fungicide. Therefore, keeping these adverse effects of chemicals in view, the present investigation was conducted to evaluate in vitro efficacy of various plant extracts against *Alternaria alternata* and *Curvularia lunata* respectively.

Materials and Methods

Collection of disease samples and isolation of pathogens

The disease samples were collected from Herbal Garden, RPCAU, Pusa, Bihar followed by isolation of pathogens were done in PDA plates. The pure culture of each fungus was obtained by hyphal tip technique. Sporulating pure culture of *Alternaria alternata* (Shakir *et al.,* 1997) and *Curvularia lunata* (Gilman, 2012) was identified on the basis of morphological features.

Plant materials used for extract preparation

Total eight plants viz Neem, Tulsi, Kalmegh, Vasaka, Papaya, Sanjeevani, Giloy and Moringa were selected to prepare extracts in order find out their efficacy against *Alternaria alternata* and *Curvularia lunata*. The plant materials were collected from MAP germplasm block, RPCAU, PUSA, Bihar. The list of selected plants was given in Table 1.

Preparation of ethanol extracts of plant materials

Fresh plant parts (leaves, stem, root) from the respective plants were collected and washed thoroughly with tap water to remove dust particles. Cleaned plant parts were dried using blotter paper to remove excess moisture followed by weighing 100g of samples of each plant and grinding along with 100 ml ethanol in 1:1 ratio. The crude extract obtained was strained through muslin cloth followed by filtered through Whatman No. 41 filter paper. These filtrates were considered as 100% and were kept at 4 °C in refrigerator for further use.

Evaluation of plant extracts against the isolated pathogens

Ethanol extracts of botanicals were tested at 5%, 10% and 15% concentration under in vitro condition. To obtain desired concentration, 5 ml, 10 ml and 15 ml standard stock solutions were poured in 95 ml, 90 ml and 85 ml of sterilized melted PDA respectively followed by pouring into Petri plates under aseptic conditions. Petri plates containing only media without plant extracts were considered as control. Thereafter, 5 mm disc of each pathogen was transferred to plant extracts treated solidified media as well as to the control plates. Each treatment was replicated three times. Inoculated plates were incubated at 28±1 °C temperature in B.O.D for one week. Percent inhibition was recorded after 7 days by using the formula given below (Bliss, 1934).

$$\text{Percent inhibition over control} = \frac{(C - T)}{C} \times 100$$

Where, C= growth of pathogen in control in mm
T= growth of pathogen in treatment in mm

Statistical analysis

Data was analysed using Analysis of Variance (Fisher and Yates, 1963) through factorial arrangements under completely randomized design (CRD). Statistical Analysis Software (SAS) was used to perform the analysis and the means were compared by using Duncan's Multiple Range Test.

Results and Discussion

Antifungal activities of eight selected plant extracts against *Alternaria alternata* and *Curvularia lunata*are presented in Table 2-3 and Figure 1-2 respectively. All the plant extracts significantly reduced mycelial growth of *Alternaria alternata* and *Curvularia lunata* with all concentrations and the effectiveness of the plant extracts increased with increased concentration. Among these plant extracts, papaya leaf extract performed best as it recorded 62.80%, 74.38% and 81.26% mycelial growth inhibition of *Alternaria alternata* at 5%, 10% and 15% concentration respectively and also showed highest (72.82%) mean

mycelial growth inhibition of *Alternaria alternata*. This was followed by kalmegh extract (60.67%), tulsi (58.44 %) and sanjeevani extract (59.73 %) extract. Both tulsi and sanjeevani extract was found to be statistically at par. Similarly, moringa (42.29%) and Neem (44.88%) extract did not differ statistically and inhibition percentage was only higher than Giloy (37.83%) and Vasaka (33.99%) extract.

All the three concentrations (5%, 10% and 15%) of papaya extract also found most effective in mycelial growth inhibition of *Curvularia lunata* i.e., 59.81%, 68.9% and 90.43% respectively. Similarly highest mean percent inhibition of mycelial growth of *Curvularia lunata* was recorded with papaya leaf extract (73.05%) followed by kalmegh extract (58.85%), neem extract (53.43%) and tulsi extract (45.61). The result also indicated that giloy extract was found least effective among all plant extracts in all three concentrations. Mean inhibition percentage of the mycelial growth of *Curvularia lunata* with giloy extract was recorded 22.49%

Present findings were supported by Suleiman (2010) as he found that highest mean percentage inhibition of mycelial growth of *Alternaria alternata* was with papaya leaf extracts. Tiijani *et al.* (2014) reported that aquous extract of papaya leaf was highly effective to inhibit radial growth of *Aspergillus flavus* i.e., 0.30 cm and 0.24 cm @ 4% and 6% concentration respectively. Study of Mbadianya *et al.* (2014) revealed that papaya leaf extract at 2.5% concentration was found effective with 53.67% mycelial growth inhibition of *Helminthosporium infestans*.

Our findings are also in close agreement with the findings of Venkateswaralu *et al.* (2013) who observed that out of 15 medicinal plants, kalmegh was found most effective as it recorded lowest mycelial weight of *Sclerotium oryzae* i.e., 150 mg, 100 mg and 70 mg at 0.5%, 1% and 2 % concentration respec-

Table 1. List of plants used for preparation of extracts

SL No	Common name	Botanical name	Part used
1	Neem	*Azadirachta indica*	Leaves
2	Tulsi	*Ocimum sanctum*	Leaves, stem
3	Kalmegh	*Andrographis paniculata*	Leaves
4	Vasaka	*Justicia adhatoda*	Leaves
5	Papaya	*Carica papaya*	Leaves
6	Sanjeevani	*Selaginella bryopteris*	leaves
7	Giloy	*Tinospora cordifolia*	Leaves, root, stem
8	Moringa	*Moringa oleifera*	Leaves

Table 2. *In vitro* screening of plant extracts on growth and percent inhibition of *Alternaria alternata* causing leaf blight of Japanese mint (*Mentha arvensis*)

Botanicals	5%		10%		15%		Mean of
	Colony growth (mm)	Percent inhibition (%)	Colony growth (mm)	Percent inhibition (%)	Colony growth (mm)	Percent inhibition (%)	Percent inhibition (%)
Neem	44.5	26.47^{lm}	36.47	39.73^{j}	19.08	68.46^{c}	44.88^{d}
Tulsi	33.3	44.96^{i}	26.8	55.72^{g}	15.33	74.66^{b}	58.44^{c}
Kalmegh	23.83	60.61^{f}	20.33	66.39^{cd}	16.33	73.00^{b}	66.67^{b}
Vasaka	46.3	23.47^{m}	42.5	29.75^{l}	31	48.76^{h}	33.99^{f}
Papaya	22.5	62.80^{ef}	15.5	74.38^{b}	11.33	81.26^{a}	72.82^{a}
Sanjeevani	27.42	54.68^{g}	24.08	60.19^{f}	21.58	64.32^{de}	59.73^{c}
Giloy	43.33	28.37^{l}	38.75	35.95^{k}	30.75	49.17^{h}	37.83^{e}
Moringa	44	27.27^{l}	33.25	45.04^{i}	27.5	54.55^{g}	42.29^{d}
Control	60.5	0	60.50	0	60.5	0	0
Factors	SE(m)		SE(d)		C.D. (@5%)		
Factor A (Plant extracts)	1.71		0.85		1.71		
Factor B (Concentration)	1.05		0.52		1.05		
Factor A×B (Plant extracts × Concentration)	2.96		1.45		2.96		

Table 3. *In vitro* screening of plant extracts on growth and percent inhibition of *Curvularia lunata* causing leaf spot of Japanese mint (*Mentha arvensis*)

Plant extracts	5%		10%		15%		Mean of
	Colony growth (mm)	Percent inhibition (%)	Colony growth (mm)	Percent inhibition (%)	Colony growth (mm)	Percent inhibition (%)	Percent inhibition (%)
Neem	19	45.46^{g}	15.5	55.50^{d}	14.17	59.33^{c}	53.43^{c}
Tulsi	20	42.58^{ghi}	19.33	44.5^{gh}	17.5	49.76^{ef}	45.61^{d}
Kalmegh	17.83	48.8^{f}	13.33	61.72^{c}	11.83	66.03^{b}	58.85^{b}
Vasaka	24.08	30.86^{k}	20.5	41.15^{hi}	16.58	52.39^{de}	41.47^{e}
Papaya	14	59.81^{c}	10.83	68.9^{b}	3.33	90.43^{a}	73.05^{a}
Sanjeevani	25.83	25.84^{lm}	22.83	34.45^{j}	13.33	61.72c	40.67^{e}
Giloy	31.83	10.05^{n}	25.08	27.99^{kl}	24.58	29.43^{kl}	22.49^{f}
Moringa	26.83	22.97^{m}	21	39.71^{i}	16.17	53.59^{d}	38.76^{e}
Control	34.83	0	34.83	0	34.83	0	0
Factors	SE(m)		SE(d)		C.D. (@5%)		
Factor A(Plant extracts)	0.61		0.86		1.74		
Factor B(Concentration)	0.37		0.53		1.06		
Factor A×B(Plant extracts × Concentration)	1.06		1.50		3.00		

tively. In contrast to present findings, it was reported by Neela *et al.* (2014) that kalmegh extract showed 85% inhibition of growth of *Fusarium* sp followed by papaya extract (89.41%) at 25% concentration.

A study conducted by Anamika and Simon (2011) revealed that neem extract showed 58.6% radial growth inhibition of *Alternaria alternata* followed by tulsi extract (54.7%). Dissanayake (2015) reported that 45% mycelial growth inhibition at 25% concentration was observed with *Justicia adhatoda* (Vasaka) extract which was one of the less effective botanicals in growth inhibition of *Fusarium proliferatum*. This finding slightly supports our present findings.

According to Bobbarala *et al.* (2009) methanolic extracts of *Andrographis paniculate* (Kalmegh) was very effective in mycelial growth inhibition of *Alternaria alternata* and *Curvularia lunata* and it supports the present findings. In contrast of our present findings, Chowdhury *et al.* (2015) observed that ethanol extract of *Azadiracta indica* at all three concentrations i.e., 5%, 10% and 15% showed 100% inhibition of mycelial growth of *Alternaria alternata* and *Curvularia lunata*. Similarly, Johora *et al.* (2022) reported that *Azadiracta indica* exhibited 100% mycelial growth inhibition of *Curvularia lunata* in 15% and 20% concentration.

Conclusion

In vitro studies reveal that the extract from papaya leaves (*Carica papaya*) was most effective in reducing radial growth of foliar pathogens of mentha i.e. *Alternaria alternata* (72.82%) and *Curvularia lunata* (73.05%) followed by kalmegh extract. Hence, further study in greenhouse as well as in field condition is recommended to re-evaluate the performance of the botanicals as sole treatment and also in combination to manage the foliar diseases of mentha. The selected extracts can also be evaluated against other foliar as well as root pathogens of mint (*Mentha arvensis*) in future.

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